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STUDIES ON THE BIOCHEMISTRY OF THE BROWN FAT AND LIVER OF HIBERNATING GOLDEN-MANTLED GROUND SQUIRRELS (CITELLUS LATIRALIS)**

UNPUBLISHED PRELIMINARY DATA

by

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ABSTRACT

Respiration rates of liver and interscapular brown fat mitochondria from control and hibernating ground squirrels (Citellus lateralis) were determined with various substrates. Oxidation of *d*-glycerophosphate and glutamate by brown fat mitochondria from hibernating animals was increased, respectively, 47% and 36% over controls. Liver mitochondria from hibernating animals showed a 50% increase over controls in oxidation of succinate, but a 34% decrease with *d*-glycerophosphate. The increased oxidative activity of brown fat, together with a 40% increase in the brown fat weight/body weight ratio, indicates that the thermogenic capability of brown fat is enhanced during hibernation.

ground squirrel

hibernation

brown fat

liver

thermogenesis

mitochondrial respiration

Running Title:

Biochemistry of brown fat and liver in squirrels.

[redacted]

INTRODUCTION:

In the hamster, mitochondria of both brown fat (2) and liver (3) undergo extensive biochemical changes in the process of cold acclimation and hibernation. During cold acclimation there are increases both in the mass of the brown fat and in its oxidative enzymatic activity (2), from which it has been adduced (4) that as in the rat (17) there is a net increase in the thermogenic capacity of the brown fat. Moreover, heat production from this tissue appears essential to the process of arousal from hibernation (12, 15).

To afford further comparisons between various hibernators, the golden-mantled ground squirrel, Citellus lateralis, has been examined during euthermia and hibernation. Results from these studies are reported in this paper.

METHODS AND MATERIALS

Golden-mantled ground squirrels (Citellus lateralis) trapped in the early fall near Big Bear, California, were divided into two groups. One was placed in a room at 2 ± 1°C for three months, and the other (control group) was kept in the animal colony room at 24 ± 2°C. All animals were exposed to 10 hours of light per day, caged individually on wood chips, with hemp for bedding, and given Purina laboratory chow pellets and water ad libitum. Using the technique described by Pengelley and Fisher (9) the progress of hibernation was followed for each animal.

Just prior to sacrifice the body temperature of each animal was taken quickly. Those in the control group were always between 36 and 39°C, and unless the temperature of a hibernating animal was below 5°C it was not considered in hibernation and was consequently not used at that time. Thus, in addition to the different environmental temperature of the two groups, control animals had a body temperature close to 37°C, while the temperatures of hibernating ones were between 3 and 5°C.

The animals were killed by decapitation, and the interscapular brown adipose tissues and livers were removed, weighed and iced quickly. Mitochondria of both tissues were then isolated according to methods described elsewhere (11) except that brown fat mitochondria were isolated in 0.44 M sucrose as suggested by Lever and Chappel (8).

Mitochondrial respiratory rates on respective substrates of β-hydroxybutyrate, α-glycerophosphate, glutamate, and succinate were determined polarographically with a Clark electrode. The mixtures were basically those of Copenhaver and Lardy (5) except for modifications described in the text.

For determination of the rates of liver mitochondrial oxidation of β-hydroxybutyrate and glutamate, the reaction mixture contained (in μ moles): phosphate buffer (pH 7.3), 24; $MgSO_4$, 22.4; cytochrome c, 0.0342; ADP*, 14.04; ATP, 5.99; NAD, 1.51; nicotinamide, 10; and substrate, 100. The total volume including 0.5 ml of mitochondrial suspension was 3 ml. Reaction mixtures for liver mitochondrial oxidation of α-glycerophosphate as well as those for mitochondria of brown fat were varied according to the enzyme system studied (Table 1); the total volume in each case was 3 ml, and the final pH, 7.3. For nitrogen determinations a standard Kjeldahl method was used, and for protein assays, the standard Folin-Ciocalteu.

Statistical evaluations were made first by comparisons of variance, and, where indicated, also by Student's t-test applied to group differences; these were considered significant at $P < 0.05$.

Results:

During the approximate two months of hibernation prior to sacrifice the hibernating squirrels sustained losses in body weight of about 20% more than the controls (Table 2). Along with this, liver weights dropped proportionately so that the mass ratio, liver weight/body weight, remained constant. Conversely, the absolute weight of the brown fat increased by about 40% and hence the mass ratio, brown fat/body weight, increased by a factor of two (figure 1). The brown fat during hibernation appeared upon dissection to be deeper in color than that of the controls, and the nitrogen content of the mitochondrial fraction was higher by some 38% (Table 3). The comparable fraction taken from liver showed no change in nitrogen.

Respiration of mitochondria (Table 4) from brown fat of the hibernating animals increased over controls in the presence of either α-glycerophosphate or glutamate but not with either succinate or β-hydroxybutyrate. In contrast, the liver mitochondria from hibernating squirrels showed an increased respiratory rate only on succinate and a somewhat decreased rate on α-glycerophosphate. On the latter substrate, respiration was very slow, i.e., of the order of 5 to 10% of that in brown fat mitochondria.

Discussion:

In these ground squirrels, as in hamsters (2) the respiration of mitochondria on α-glycerophosphate was low in systems from liver but relatively high in those from brown fat. The significance of this may lie in the calorigenic potential which apparently derives from the oxidation of α-glycerophosphate through cytochrome c (7), by a poorly phosphorylating pathway (cf. 6, 14). A similar thermogenic effector action may also occur in brown fat of other species during cold acclimation (13, 17) and in the arousal response of the hibernator (12, 15, 17). Moreover, the level of activity over that in liver would suggest a qualitative difference in the major pathways of heat production employed by these respective tissues. Thus in cold-acclimated hamsters, brown fat mitochondria failed to show increased succinoxidase activity. In the liver of ground squirrels however, the greater activity of mitochondria from the hibernating animals over that of the controls is reminiscent of similar differences observed in succinic dehydrogenase activity among various cold treated rodents (3, 13, 14). Again, while glutamate was oxidized most rapidly in the liver systems, only the brown fat mitochondria showed a significant increase of activity in the hibernating animals.

Some impression of the relative efficiency of respiratory coupling in these tissues may be gained from comparison of the QO_2 obtained with liver and brown fat on each substrate with that on β-hydroxybutyrate (Table 4) after each is normalized for theoretical P/O differences. On this basis, the difference between these normalized values and those obtained empirically represents roughly the increment of respiration which evidently did not contribute to phosphorylation. If this difference is assumed to represent the extra caloric yield in each case, it is evident from the table that substantially greater heat yields appear in the systems from hibernating animals than in controls for succinate utilization in liver and for both succinate and d-glycerophosphate utilization in brown fat.

The fact that the liver size decreases in hibernating ground squirrels but increases in cold-acclimating hamsters, is further evidence of the difference in patterns of the physiological responses observed in these two species.

The nitrogen/gm dry weight of brown fat mitochondria of the hibernating ground squirrel (Table 3) is enough above the control value to suggest that a wholesale change in the protein content has occurred in the mitochondria; however, at least some of the observable nitrogen increase may have resulted from changes in mitochondrial RNA, or nitrogen containing cofactors.

The data seem to show that the intracellular physiological changes, as well as the gross weight change in the brown fat observed in hibernating animals, are in fact due to the cold environment at which they were kept.

These internal changes probably contribute greatly to the cause of the observed phenomenon in this species that a cold environment speeds the onset of hibernation but is not necessary for it to take place (10). It is important to note that the control animals kept at 24°C showed almost no signs of hibernating, which is at variance to previous research on this species (10). Furthermore, the group at 2°C hibernated later than would have been predicted from previous work. The discrepencies are probably due to physiological differences in the two subspecies concerned. The subspecies used in this research was bernardinus found at the most southern point (latitude 34 N) of the species' range, while the previous work was on tescorum found at the most northern point (latitude 53 N). An animal at the northern limit would be well adapted to its harsher environment if it hibernated early in the season, while an animal at the southern limit would be well adapted if it hibernated later, or perhaps not at all if the mean environmental temperature was warm enough. A cold environment has been shown here to affect the cellular biochemistry in such a way as to apparently prepare the animal rapidly and efficiently for entrance into hibernation. One can see therefore that from an evolutionary view, this is a good mechanism for ensuring intraspecific adaptation in the form of a differential time of entrance into hibernation in a species with a wide range of latitude.

Summary:

Studies were made on the effects of cold acclimation culminating in hibernation, on the weight and the mitochondrial nitrogen content and enzymatic activity of brown fat and liver of ground squirrels (Citellus

lateralis). The hibernating animals were living at a room temperature of 2 ±1°C. The body and liver weights of the hibernating animals remained in constant ratio as both were approximately 20% lower than in controls; but as concurrently the brown fat was 40% greater, its ratio to body weight of the hibernating animals was double that of the control. Brown fat mitochondria from the hibernating squirrels showed significant increases in respiration associated with α-glycerophosphate but not with β-hydroxybutyrate or glutamate. In the hibernating group succinate oxidation was increased in mitochondrial systems from both brown fat and liver but significantly so only in the latter. Nitrogen content per gm (dry weight) of mitochondria was unchanged in liver but significantly higher in the mitochondria of brown fat from hibernating squirrels. It is concluded that a cold environment increases both the size and the thermogenic potential of the brown fat in the ground squirrel Citellus lateralis, which enhances the animal's preparation for hibernation.

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Footnotes

* ADP = adenosine diphosphate

ATP = adenosine triphosphate

NAD = nicotinamide adenine dinucleotide

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TABLE I. Reaction mixtures for oxidase assays of brown fat mitochondria.

	Substrate and Oxidase System			
Component*	Succinate	Glutamate	β-hydroxybutyrate	α-glycerophosphate**
Phosphate	48.00 μ moles	40.00 μ moles	20.00 μ moles	44.00 μ moles
ADP	14.04	14.04	14.04	14.04
ATP	5.99	5.99	5.99	5.99
NAD	---	3.77	3.77	---
Substrate	50.00	50.00	50.00	100.00

* Additionally, all systems contained 22.4 $\mu\frac{\text{moles}}{\text{M}}$ $MgSO_4$ and 0.0342 $\mu\frac{\text{moles}}{\text{M}}$ Cytochrome c.

** Reaction mixture used for both liver and brown fat.

TABLE 2. Body weights (gm) and weights of brown fat and liver in gm and percent of body weights in hibernating and control ground squirrels.

Experimental Group	n	Body weight	Brown fat weight	Brown fat weight (%)	Liver weight	Liver weight (%)
Hibernating	(16)	194.54* ± 10.52	2.54 ± 0.10	1.0 ± 0.03	5.98 ± 0.35	3.0 ± 0.10
Control	(18)	236.90 ± 8.04	1.55 ± 0.16	0.65 ± 0.04	7.17 ± 0.31	3.0 ± 0.13
P diff ≤		0.01	0.001	0.001	0.02	1.0

* Means ± S.E.

TABLE 3. Nitrogen content (mg) per gm dry weight of mitochondria from tissues of hibernating and control ground squirrels.

Mg N/gm dry mitochondria

Group	n	Liver	Brown fat
Hibernating	(14)	30.47 ± 3.89	22.25 ± 1.87
Control	(14)	34.46 ± 2.45	16.04 ± 1.23
P diff ≤		0.2	0.01

TABLE 4. Effect of hibernation on respiration of liver and brown fat mitochondria with various substrates *in vivo*.

Tissue source	Substrate and Respiration*			
	β-hydroxybutyrate	glutamate	α-glycerophosphate	succinate
Hibernating liver	25.7 ± 3.4	31.7 ± 3.4	4.4 ±0.32	58.4 ± 6.3 38.5 19.9 ***
Control liver	27.5 ± 2.7	33.7 ± 2.9	6.7 ±0.28**	38.8 ± 3.8** 41.2 -2.4
Hibernating brown fat	15.8 ± 2.5	11.6 ± 0.80	89.4 ± 1.7 47.4 42.0	78.6 ± 7.3 23.8 54.8
Control brown fat	23.1 ± 4.1	8.5 ± 0.75**	61.0 ± 2.5† 69.5 -8.5	63.0 ± 5.9 34.7 28.3

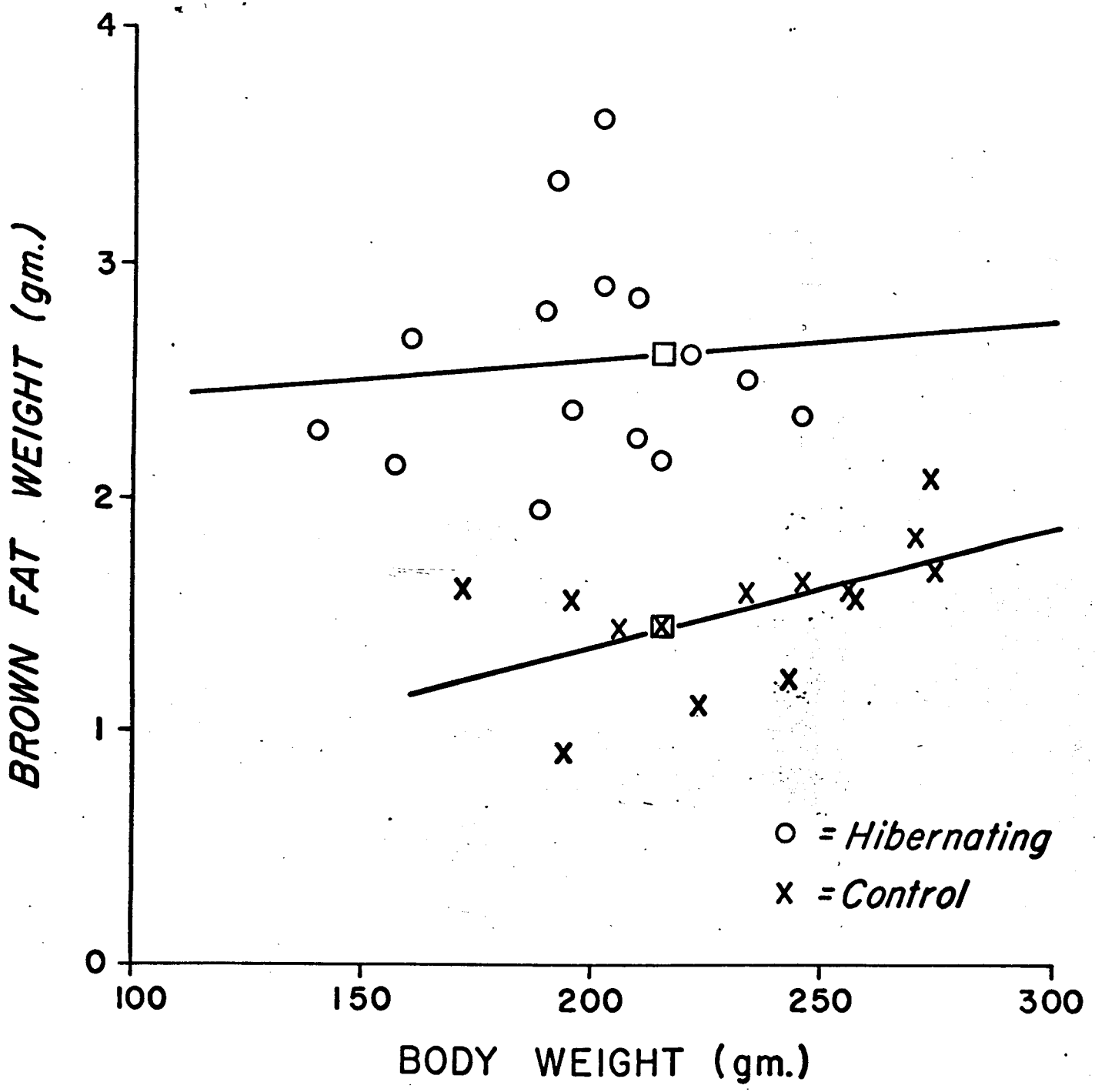
* µl O_2/mg protein and hour (mean ± S.E.; n = 14)

** $P \leq 0.05 - 0.02$; †$P \leq 0.001$

*** Difference between observed respiration and that calculated for same ∼P fixation as expected for β-hydroxybutyrate. P/O values assumed respectively for β-hydroxybutyrate and α-glycerophosphate and succinate were: 3,1,2.

Legends for figures

Figure 1: Regression lines of brown fat weight *vs* body weight for control and hibernating ground squirrels. When tested statistically at a body weight of 215 gm, weights of brown fat from control and hibernating animals differ significantly from each other ($p < 0.001$).


BROWN FAT WEIGHT (gm.)
4
3
2
1
0
100
150
200
250
300
BODY WEIGHT (gm.)
O = Hibernating
X = Control